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Life history and Ecology of *Speyeria adiaste clemencei* (Comstock, 1925) (Lepidoptera: Nymphalidae)

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Abstract. In this paper we describe the life history and ecology of an endemic and declining California butterfly subspecies *Speyeria adiaste clemencei* (Comstock, 1925) from Chews Ridge, Monterey Co., CA. Despite its limited range, declining numbers, and one of the three *S. adiaste* subspecies already being extinct, a complete life history of this species has not been published. Our observations set the groundwork for future studies assisting in the conservation of this species. *S. a. clemencei* can be successfully reared on commercially available *Viola* spp., facilitating captive rearing for restoration. Larvae of *S. adiaste* can be distinguished morphologically from sympatric *S. callippe* and *S. coronis* larvae based upon coloration of the dorsal and dorsolateral scoli, head capsule coloration, and coloration of setae, facilitating identification in the field. We also document differences in adult behavior between the sexes and describe a shift in nectar source use during the observation period. We suggest that adequate access to nectar sources throughout the flight period, especially during drought years, as well as host plant density and distribution, are critical aspects for maintaining viable *S. a. clemencei* populations. These and other aspects of the ecology of *S. a. clemencei* warrant further study in order to better understand this imperiled species.

Keywords: larval ecology, immature stages, adult resource, endemic, *Viola purpurea*

Introduction

Butterflies are among the most well-known insects (New, 1997), and although early naturalists recognized the potential utility of immature stages in resolving taxonomic issues in butterflies (Müller, 1886; Edwards, 1877), the immature stages have been relatively understudied. Freitas and Brown (2004) have implicated various factors for this asymmetry, including a lack of larval specimens available in museums, low interest by lepidopterists, and the inherent difficulties in completing necessary fieldwork. Recent work on the morphological study and ecology of immature stages has proven valuable in resolving species boundaries, taxonomic issues, and cases of strong convergent evolution of adult phenotypes (Hill *et al.*, 2012; James & Nunnallee, 2011; Aiello, 2006; Freitas & Brown, 2004; Pech *et al.*, 2004; Brown & Freitas, 1994; Willmott & Freitas, 2006). Immature stages have also seen increasing attention in phylogenetic studies, but these often focus on a limited number of taxa (Penz *et al.*, 2013).

In addition to contributing to lepidopteran systematics, understanding larval ecology is crucial to the conservation of endangered species. This is particularly important for *Speyeria* butterflies, which have been in decline throughout the United States for the past 200 years (Hammond & McCorkle, 1983). For example, Bierzychudek *et al.* (2009) studied larval movement of endangered *Speyeria zerene hippolyta* to elucidate host-finding behavior. These caterpillars were unable to differentiate between their host plant and non-host plants at a distance of three centimeters, highlighting the need to focus on host plant density and spatial distribution in restoration efforts. *Speyeria zerene hippolyta* has recovered from near extinction, as a result of tree clearing, thatch removal, and mowing

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that successfully increased abundance of the *Viola adunca* host plant (Hammond, 1987, 1988, 1989; Hammond & McCorkle, 1991). Studying the effects of mowing regimes has also provided important insight into effective management practices in other butterfly species (Konvicka *et al.*, 2003; Johst *et al.*, 2006).

Studying larval ecology of *Speyeria* is of particular interest in California, where four of the thirteen federally-listed endangered or threatened butterflies are *Speyeria* taxa (USFWS, 2014). In addition to these four, an additional species, *S. adiaste*, has reportedly also been declining (Glassberg, 2001; Opler & Wright, 1999; Shapiro & Manolis, 2007; Scott, 1986; Kaufman & Brock, 2003). In contrast to the four federally-listed *Speyeria* taxa, the non-listed *S. adiaste* is the only *Speyeria* species endemic to California, and the only *Speyeria* species to have an entire subspecies population go extinct (with the possible exception of *Speyeria zerene myrtleae*, see Shapiro & Manolis, 2007). The unlisted status of *S. adiaste* has not gone unnoticed as there have been two petitions to have it listed, one in 1992 and the other in 2010 (USFWS, 2011). Despite its relatively narrow range and declining numbers, a complete life history for this species has not been published. According to Howe (1975), the life history for *S. a. atossa* was described by J. A. Comstock and C. M. Dammers and water color paintings were done by C. M. Dammers. Comstock and Dammers (1931) provide a description of *S. a. atossa*, but this is limited to the ultimate (6^{th}) instar, and the larva was not illustrated. Howe (1975) reports that the Dammers paintings were deposited in the Natural History Museum of Los Angeles County, but a search by museum staff was unsuccessful (R. P. Hulser, Chief Librarian pers. comm.). The lack of published information on this species, combined with its apparent decline and attempts to obtain listing status for *S. adiaste* motivated our current study of the life history and ecology of *Speyeria adiaste clemencei* at a well-known, long standing, and accessible population on Chews Ridge in Monterey Co, CA.

Materials and methods

Observations in this study were focused on the population at Chews Ridge, Monterey County, CA (N 36.31336°; W 121.57323°, 1500 m elevation). The habitat of *S. a. clemencei* at Chews Ridge is characterized as mixed oak-pine woodland. For identification of the *Viola* host plant and nectar sources found at Chews Ridge, we consulted Baldwin *et al.* (2012) and Matthews (2006). Observations on adult behavior were recorded during the course of adult censuses and a mark recapture study during the summers of 2012 and 2013. The behavior of each butterfly was recorded at the moment of capture. Egg duration was obtained by collecting eggs observed being laid by females in the field, which typically occurs in July and August. To obtain larvae for morphological study, wild-caught females were brought to the lab and placed in large brown paper grocery bags with dried *Viola* spp. similar to the methods of Mattoon *et al.* (1971). The dried host plant used to stimulate oviposition was a haphazard mixture of *V. papilionacea*, *V. pedunculata* and commercially available pansies (*Viola* spp.). We waited until late in the flight season to obtain worn females in an effort to minimize disturbance on the natural population. The females were fed one to two times per day on a 7% honey water solution soaked onto a sponge. They were allowed to feed until they were satiated. Females were preserved for genetic studies and the bag was closed when oviposition ceased. After two weeks the hatched larvae were removed from the bag, counted, and stored in wood blocks at 4°C for approximately four months. The wood blocks were one-inch by one-inch cubes of Douglas fir with a half-inch hole drilled completely through the center. The larval chamber was covered by fine mesh and stapled shut. The blocks were placed on dampened paper towel in an open plastic container. The blocks were misted semi-daily, and the paper towels changed frequently to prevent the accumulation of mold.

To break winter diapause of the first instars, each larva was placed into a one ounce opaque plastic Solo brand sauce cup lined with slightly damp paper towel that was re-wetted each day. Cups were placed under constant lighting approximately 14 inches below two 40W fluorescent lamps, and the larvae were stimulated twice daily using a small paintbrush. Larvae were fed commercially available pansies (*Viola* spp.) or *V. papilionacea* with the leaf petiole wrapped with damp paper towel. Larvae were fed *ad-libitum*. Larval head capsules and shed exuviate were collected at each molt and preserved in 70% ethanol. Notes on their behavior and morphology were taken daily. Photos of each stage were taken using a Nikon D80 camera, and 105 mm macro lens with extension tubes. Upon reaching the 5^{th} instar, larvae were transferred to larger two ounce plastic cups (Solo). Pupation occurred in these larger cups and pupae were subsequently attached to the lids of tall 10-ounce clear plastic drinking cups lined with paper towel for eclosion. Upon eclosion, adults were transferred to a 12-inch mesh cube to allow wings to harden before being frozen for preservation. Larvae of each instar were preserved in 70 % ethanol for morphological study. Images of head capsules were obtained using

a Leica S8 APO stereomicroscope with an attached Leica DFC295 camera. Leica Application Suite version 3.8 was used to measure head capsule images. Head capsule width was measured horizontally at its widest part, just above the dorsal-most stemmata in frontal view. Scanning electron micrographs of 1st instar morphology were taken with a Hitachi S-2600N scanning electron microscope (Hitachi High Technologies, Tokyo, Japan). The sample was first dried in an EMS 850 critical point dryer (Electron Microscopy Sciences, Hatfield, PA), using acetone as the intermediate solvent, and was then coated with gold in a Pelco SC-7 sputter coater (Ted Pella, Inc., Redding, CA), following manufacturer's protocols.

For the description of the larval stages, only one half of each segment was described. For each instar, at least two preserved specimens were used for morphological descriptions, supplemented with photos and descriptions from living larvae. When describing the larval scoli, we refer to three "rows" of scoli on each side of the midline. There is a dorsal row extending the length of the body, a dorsolateral row extending the length of the body, and a lateral row extending from A1 posteriorly. 1st instar chaetotaxy follows Hinton (1946), Kitching (1984), and Scott (1986).

To investigate the potential for field identification of larvae, we compared the larval color pattern in 2nd through 6th instars for the three sympatric *Speyeria* species found on Chews Ridge. The following 14 characters were used: proleg color, thoracic leg color, head capsule color, presence/absence of tan/brown patches on the head capsule, color of setae on scoli, color and thickness of dorsal line, body color, size and number of black body patches, presence of a lateral mottled gray line between dorsal and dorsolateral scoli, T1-A8 dorsal scoli color, T2-A8 lateral scoli color, A1-A8 dorsolateral scoli color, and the coloration of the A9 scoli and A10 scoli.

The observed behavior of adults was classified into five categories: chasing, search flight, direct flight, perching, and nectaring. These behaviors were distinct and could be unambiguously determined in the field. Chasing was a distinctly male behavior in which males would fly after other butterflies, insects, or birds. Individuals chasing each other would often circle one another rapidly flying upwards. Search flight was characterized as flying back and forth in an area at relatively low velocity, with no apparent overall direction. Search flight was clearly distinct from chasing and direct flight, and was used as a category for both males and females since it could be quickly categorized during mark recapture studies. However, it was clear that search flight behavior was distinct for males and females. Male search flight was characterized by flying back and forth in a small area, often moving in one direction then immediately circling back. The males would also occasionally land among vegetation for short periods before continuing their search flight. They seemed to be in search of females and/or nectar. Females in search flight flew much lower to the ground, and they often landed among vegetation and proceeded to walk around for short periods, before flying again and repeating the landing/walking sequence several times. This behavior was presumably an attempt to locate their host plant. Direct flight referred to a butterfly flying in a more-or-less straight line in one direction at a relatively high velocity without stopping or circling back in the opposite direction. Perching individuals were those seen sitting on the ground or vegetation, such as small shrubs or oak leaves, with wings opened or closed. Differences in nectar use and adult behavior were tested using Fisher's Exact Test (fisher.test) in R (version 3.0.2).

Results

Egg (Fig. 1A). Two eggs were measured. Egg 1: height (base to apex) = 0.96 mm, max width = 0.85 mm, height to width ratio = 1.1; egg 2: height = 0.94 mm, max width = 0.94 mm, height to width ratio = 1.0. Both eggs hatched in 14 days. Eggs are pale yellow-straw colored when laid, turning to brown after one to two days. The larval head capsule is visible within the egg the day before hatching. Egg is ovoid but relatively wide at base and tapered strongly at apex. Egg is about as tall as it is wide, and widest in basal third. Egg is sculptured with vertical ridges and resulting troughs; ridges occasionally bifurcate near base and are crossed at regular intervals by horizontal lines. Number of ova per female per day and total number of ova per female is unknown. One individual female laid 70 eggs in the lab during this study, but it is expected that females are able to lay up to a few hundred eggs in total, similar to that reported for other *Speyeria* species by James and Nunnallee (2011).

1st instar (Figs. 1B, 1C). Mean duration after ending diapause: 12 days (n = 18, range = 6-27 days, s.d. = 5.2). Mean head capsule width: 0.41 mm (n = 17, s.d. = 0.013). Head – Head capsule is sclerotized, dark brown to black with many brown setae. The setae protrude from a glossy dark brown to black sclerotized round base and have serrate projections. The stemmata appear black; the area enclosed by the ring of stemmata is a darker black on preserved specimens compared to the rest of the head capsule. Head capsule chaetotaxy is provided in Fig. 2. Body – Overall body pale brown freckled with many brown spots. The body is covered in setae and a 1st instar setal map is provided in Fig. 1B. Dorsal setae have bulbous tips (series D, XD, SD, and L), except for SD1 and L1 on A10, and setae have serrate projections. The body setae have conical bases. T1: Dorsally there is a dark brown to black sclerotized prothoracic shield located just posterior to the head capsule. The prothoracic shield has eight dark brown setae, four on each side of the midline (XD1-XD2, D1-D2). Laterally there are two dark brown sclerotized patches, with one (SD) superior to the other (L1-L2). Both of these are ovoid with two setae. There is a brown spiracle located posterior to the inferior-most lateral patch (L1-L2) that has a round opening at the apex of a conical protruding base, unlike the spiracles of the abdominal segments which are smaller and more flat. Ventrolaterally there is one dark brown sclerotized patch with two setae (SV1-SV2) protruding

from it. T2-T3: Dorsal to dorsolaterally there are three dark brown sclerotized patches (D1, D2, SD). The superior-most patch (D1) has two setae protruding from it whereas D2 and SD have only one seta. Laterally there is a dark brown sclerotized patch (L) with three setae. Ventrolaterally there is a dark brown sclerotized patch (SV1) with one seta. The thoracic legs (T1-T3) are heavily sclerotized, dark brown to black in color like the sclerotized body patches, with many setae protruding from the segmented areas of the legs. A1-A2: Dorsal to dorsolaterally there are three dark brown sclerotized patches (D1, D2, SD) that are similar in size and shape with the exception that the inferior-most of these three patches (SD) has one seta instead of two as seen on SD of T2-T3. Laterally there is an ovoid, dark brown sclerotized patch (L) with four setae. A small, ovoid, dark brown to black spiracle is located superior to L. The dark colored abdominal (A1-A8) spiracles stand out from the background body color. The spiracles on A3-A8 have the same morphology as on A1-A2. Ventrolaterally on A1-A2 there is a sclerotized patch (SV1) with one seta protruding from it. Ventrally on A1 there are two (one on each side of midline), very small, light brown sclerotized patches (P6), each with one small seta. Ventrally on A2 there are four (two on each side of midline) very small light brown sclerotized patches (P6 and V1) with one small seta. A3-A6: Dorsal to dorsolaterally there are three sclerotized patches (D1, D1, SD) that have the same morphology as the D1, D2, SD patches described in T2-T3. Laterally there is a sclerotized patch (L) with the same morphology as described for the lateral L patch in A1-A2. Ventrolaterally segments A3-A6 are devoid of sclerotized patches, but there are sclerotized patches on the prolegs. The prolegs are a pale brown, very similar in color to the body. Laterally each proleg has a dark brown to black sclerotized patch adorned with two setae (P2 and P4). A7-A8: Dorsal to dorsolaterally there are three patches (D1, D2, SD) with the same morphology as described for the D1-D2-SD patches in T2-T3. Laterally there is a sclerotized patch (L) with the same morphology as described for the lateral patch (L) in A1-A2. Ventrolaterally A7 and A8 both have one sclerotized patch (SV1) that is small, with one seta. Ventrally A7 has two (one on each side of midline) small, brown, ovoid sclerotized patches (P6) with one seta each, whereas A8 is devoid of patches ventrally. A9: Dorsal to dorsolaterally the morphology is the same as described for T2-T3. Laterally there is a dark brown sclerotized patch with one seta (L1). Ventrolaterally there is a brown sclerotized patch with one seta (SV1). A10: Dorsally there is a large dark brown to black sclerotized suranal plate with ten setae (five on each side of the midline) protruding from it (D1-D2, SD1, L1). There are no sclerotized patches in the lateral and ventrolateral regions. Ventrally there are ten small sclerotized patches some of which bear a single seta. These ten patches are arranged as two rows of four patches followed by a posterior-most row of two. The anal prolegs have large dark brown to black sclerotized patches laterally that bear many setae (P1-P4).

2nd instar (Fig. 1D). Mean duration: 6.7 days (n = 16, range = 4 to 9 days, s.d. = 1.4). Mean head capsule width = 0.60 mm (n = 17, s.d. = 0.026). Head – Very dark brown to black, numerous setae with two different general size classes. The smaller setae are more densely distributed near the mouthparts while the larger setae (~2x larger) are found superior to the stemmata on the head capsule. Setae are dark brown to black and have dark brown to black glossy cylindrical bases with bulbous ends. Stemmata appear black. Body – Overall the body is dark brown with cream colored mottling. Dorsally two cream colored stripes run the length of the larva. T1: The dorsal scolus projects overhead, particularly at rest. A second scolus lies dorsolaterally in the extreme posterior of T1 at border with T2. The dorsal scolus is entirely very dark brown to black. Superior to the dorsal scolus there is a black sclerotized prothoracic shield. Dorsolaterally there is a black sclerotized patch, roughly oval in shape with two setae. These setae are dark brown to black with conical bases and bulbous tips as seen on the head capsule and rest of the body. Laterally there is a black sclerotized patch that is more triangular in shape, and bears four setae. Just posterior to the lateral patch is the spiracle, with a black base and circular opening. Ventrolaterally there is a very dark brown to black oval-shaped sclerotized patch bearing three setae. T2-T3: Dorsal scolus colored as on T1. Dorsolaterally there are two black sclerotized patches, the triangular shaped anterior patch has four setae, and the posterior patch has two setae. Ventrolaterally there are three sclerotized patches; two larger patches with one superior to the other with four setae each, and a very small patch with one seta, located more anteriorly and superior to the thoracic legs. The thoracic legs contain sclerotized patches laterally. The sclerotized portions of the legs are black with non-sclerotized portions dark brown. A1-A2: Dorsal scolus as in previous segments. Dorsolaterally there is an entirely dark brown to black scolus roughly in line with the dorsolateral scoli of T2 and T3, but just slightly superior. Just inferior to this dorsolateral scolus, there is a spiracle that is much smaller than the spiracle found on T1. All of the abdominal segment spiracles are similar in size, shape, and color. They are ovoid in shape with a circular opening. Laterally there is a scolus with mixed coloration. The basal third is bright yellow, the middle third is a brownish-yellow and the distal third darkens to a black tip. Ventrolaterally there are two black sclerotized patches, one superior to the other, and both with three setae. The color of these patches is the same as all sclerotized body patches (dark brown to black), and the setae here are also dark brown to black. A3-A6: Same as A1-A2, except that there is only one dark brown to black sclerotized patch with two to three setae with 1-2 setae lateral to this patch. The prolegs are yellowish-brown in color and bear many small setae. A7-A8: Same as A3-A6, except that ventrolaterally there are two small sclerotized patches, both of which have two setae on A7, and one seta each on A8. A9: Dorsally the same as segments A7-A8. Dorsolaterally there is a sclerotized patch bearing one seta. Ventrolaterally there is one small sclerotized patch with one seta. A10: Dorsally the same as A9, but the scolus is shifted slightly inferior following the curvature of the segment. There is a black sclerotized suranal plate found in between the two dorsal scoli. Ventrolaterally there are several small sclerotized patches surrounding the anus and anal prolegs.

3rd instar (Fig. 1E). Mean duration: 6.6 days (n = 14, range = 4 to 12 days, s.d. = 1.8). Mean head capsule width = 0.91 mm (n = 17, s.d. = 0.034). The 3rd instar is a darker color with more black and less dark brown than the 2nd instar. The cream mottling on the body is much more apparent than on the 2nd instar. The arrangement and number of sclerotized patches and scoli are the same as in the 2nd instar. However there is an important change to coloration of some scoli. The orange coloration on the lateral scoli of A1-A8 is much brighter compared to the 2nd instar where the scoli gradually change from orange-brown to orange. The coloring of the rest of the scoli is the same; the dorsal scoli remain very dark brown to black throughout. The dorsal twin stripes are more apparent in the 3rd instar compared to the 2nd instar.

4th instar (Fig. 1F). Mean duration: 5.8 days (n = 12, range = 5 to 7 days, s.d. = 0.75). Mean head capsule width = 1.3 mm (n = 17, s.d. = 0.057). The arrangement and number of sclerotized patches and scoli are the same as in the 3rd instar. This instar has a more prominent orange coloration in the inferior-most lateral scoli of A1-A8 (due to increase in size). On the head capsule, superior to the stemmata and located to the left and right of the epicranial suture, are dark brown patches, sometimes with a stripe-like appearance. There is variation in the coloration of these patches with some individuals displaying tan to light brown colored patches on their head capsules. Similar small patches are found just superior to the base of both antennae. The mottling on the body is more apparent compared to 3rd instars, where the bright mottling near the base of the orange lateral scoli appears almost stripe-like when viewed from a distance with the naked eye.

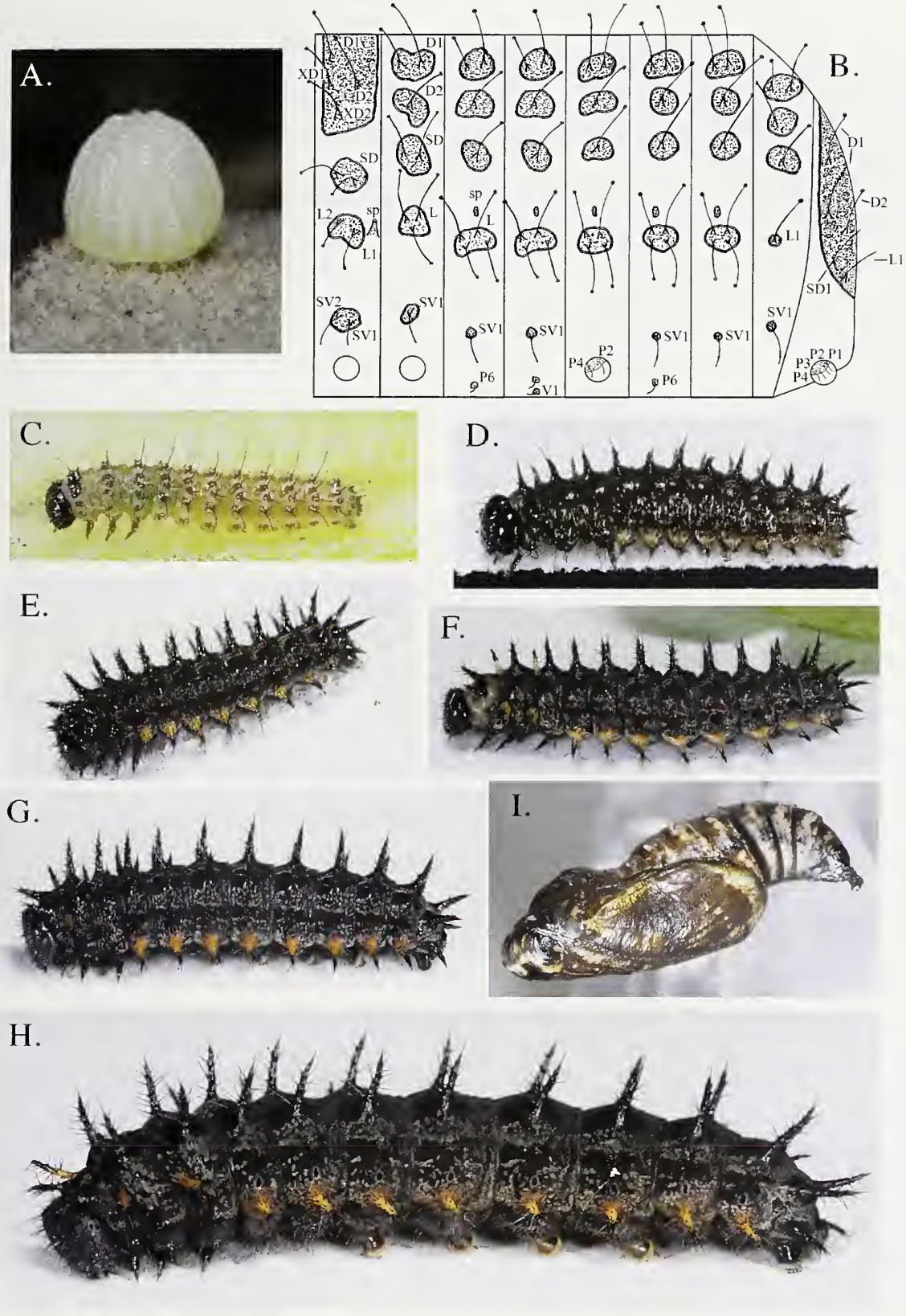

Figure 1. Immature stages of *S. adiaste clemencei*. (A) Egg (B) First instar setal map. Names of setae/patches belonging to the same row are indicated in the most anterior segment. (C) First instar (D) Second instar (E) Third instar (F) Fourth instar (mid-molt) (G) Fifth instar (H) Sixth instar (I) Pupa. Note: Images represent different individuals from the same brood.

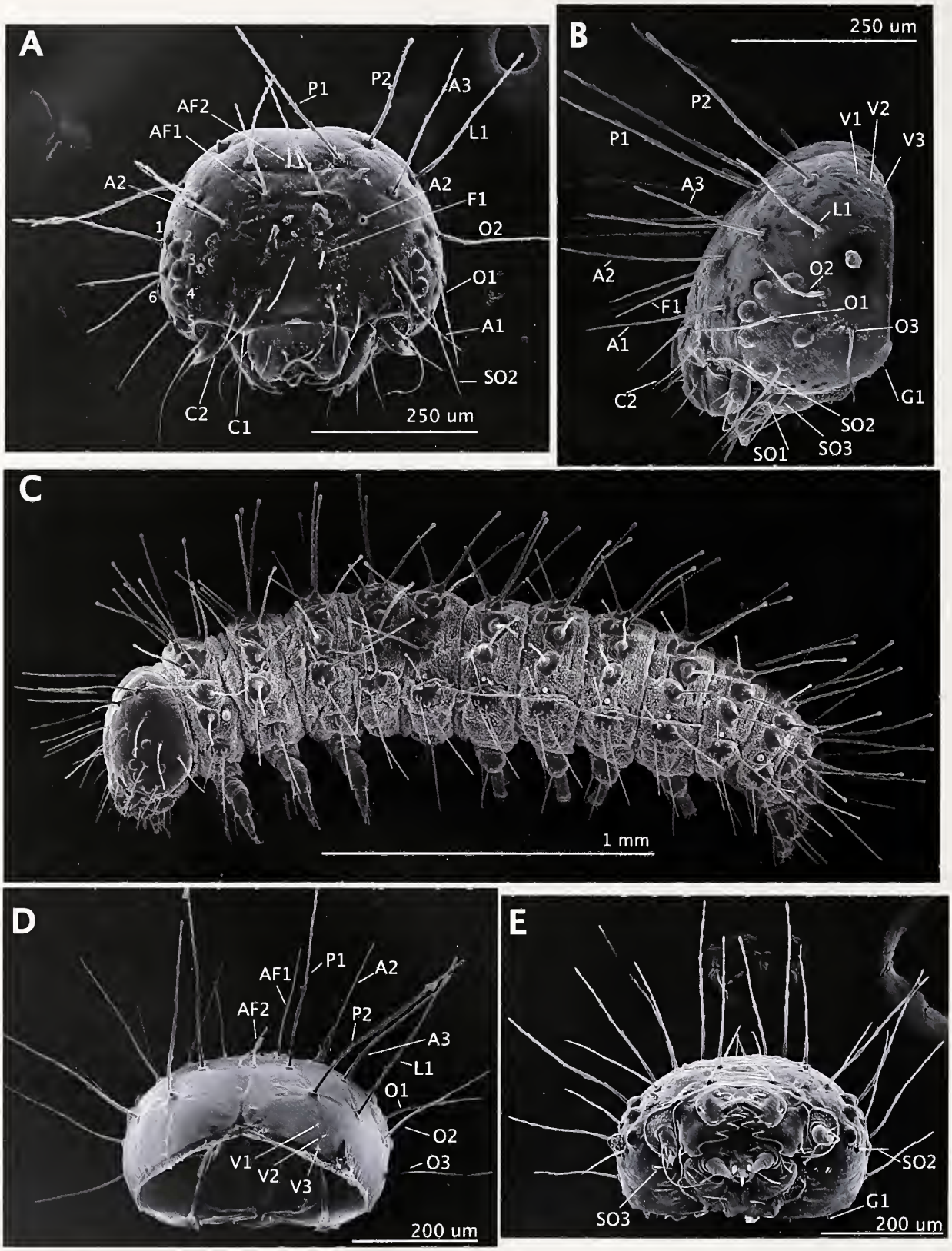

Figure 2. *S. a. clemencei* 1st instar head capsule and larval SEM images. (A) Head capsule - frontal view (Note: There is a broken seta between C1 and F1). (B) Head capsule - lateral view. (Note: round object superior to O2 seta is dirt) (C) 1st instar (D) Head capsule - dorsal view (E) Head capsule - ventral view.

5th instar (Fig. 1G). Duration = 5.5 days (n = 11, range = 4 to 7 days, s.d. = 0.82). Mean head capsule width = 2.0 mm (n = 17, s.d. = 0.083). The 5th instar is the same as previous instar except for an increase in body size.

6th instar (Fig. 1H). Mean duration: 10 days (n = 10, range = 8 to 15 days, s.d. = 2.0). Mean head capsule width = 2.9 mm (n = 5, s.d. = 0.091). Head – Head capsule is well-sclerotized, colored black and bears many black setae. The bulbed setae are either black with dark brown tips or entirely black. The setae on the head capsule are in two size classes, with the smaller setae being approximately one-fourth to one-half of the length of the larger setae. The larger setae tend to have brown tips. The majority of the larger setae are located dorsally. The setae have glossy black sclerotized round patches at the base. The brown stripe-like patches on the head capsule are more easily visible and are brighter than in previous instars. The stemmata appear black. Body – Body color is mottled charcoal gray and black. Twin cream-colored stripes run the length of the larvae dorsally. A relatively distinct mottled gray band lies between the dorsolateral and lateral scoli along the entire length of the larva. The prominence of the gray band is somewhat variable but strongest in the middle segments. T1: The dorsal scolus faces anteriorly and projects over the head, whereas the dorsal scoli of the other segments are upright and approximately perpendicular to the long axis of the body. Surrounding the dorsal scolus, there is a rectangular black patch, approximately two-thirds of which lies anterior of the scolus and one-third lies posterior of the scolus. The basal 33-40% of the dorsal scolus has a dark brown color that turns to black in the upper 60-67%. The dorsal scolus has approximately twenty setae along its shaft. The setae have bulbous ends and are of equal length. The scolus terminates with a ring of three to four setae, which are shorter than the rest of the setae on the scolus, and a single terminal mid-length seta. The setae are black in color and at the base of each seta is a glossy-black patch where it extends out of the scolus. Superior to the dorsal scolus is the prothoracic shield, which is less prominent in this instar than in younger instars. Laterally there are two dark brown to black sclerotized patches. The superior-most lateral patch is circular in shape and bears six black setae. Directly inferior to this patch, is the second somewhat larger and more triangular shaped patch with approximately ten black setae protruding from it. Posterior to the triangular sclerotized patch is the first spiracle. This spiracle is slightly larger than the rest of the spiracles on A1-A8. The ovoid T1 spiracle is black which stands out against the background body color, which is not as dark. Ventrolaterally there is a small triangular sclerotized patch with five to six black setae protruding from it. Ventrally and anterior to the two thoracic legs is the eversible neck gland, which emits a foul odor when the caterpillar is disturbed. The thoracic legs are dark brown to black and well-sclerotized. T2-T3: The dorsal scolus is the same color as the T1 dorsal scolus and points vertically, perpendicular to the long axis of the body. Dorsolaterally there is a small black sclerotized patch with approximately ten setae protruding from it. Laterally there is a black sclerotized patch with approximately ten setae protruding from it. Anterior and slightly inferior to the dorsolateral patch, there is a scolus located at the boundary between segments T1 and T2, and an identical scolus lies between T2 and T3 in the same plane. The basal 60% of these scoli are orange with the apical 40% black. Ventrolaterally there are three ovoid black sclerotized patches located just above the thoracic legs. One of these is smaller and more anterior and all three have five to ten setae protruding from them. The thoracic legs are the same as on T1. A1-A2: The dorsal scolus on each of these segments is entirely black. Unlike T2-T3, there is not a dorsolateral sclerotized patch. Dorsolaterally there is a scolus with the basal 25-33% colored dark brown, middle 33-50% orange-brown, with the remaining distal portion black. Laterally there is a second scolus just inferior to the spiracle, with the basal 80% bright orange and apical 20% black. There is variation in the coloration of that lateral scolus; some individuals go from the bright orange to a more yellowish or tan color above 20% basally as opposed to the more uniform basal 80% bright orange, apically the black is constant. The spiracle is ovoid and black. Ventrolaterally there are two roughly ovoid black sclerotized patches, one superior to the other, and each bearing approximately eight setae. Ventrolaterally and ventrally there are numerous scattered setae. A3-A6: Same as A1-A2, except that ventrolaterally there is only one black sclerotized patch instead of two. The prolegs are pale orange to tan, with a dark brown to black elongate sclerotized patch laterally bearing numerous setae. A7: Same as A1-A2, except the two black sclerotized patches are relatively smaller. A8: Same as A3-A6, except that the ventrolateral black sclerotized patch is smaller and located more inferiorly. A9: Dorsally the same as A1-A8. Laterally there is a black sclerotized patch with four setae. Ventrolaterally there is a black sclerotized patch with three setae. A10: The only scolus in this segment is located laterally and is completely dark, resembling the dorsal scoli of segments A1-A9. The posterior portion of A10 contains a black sclerotized suranal plate (above the anus). Posterior to the anus is the anal proleg that is colored the same as the A3-A6 prolegs but has a relatively larger basal black sclerotized patch laterally which bears numerous setae.

Pupa (Fig. 1I). Mean duration: 15 days (n = 8, range = 14 to 17 days, s.d. = 1.0). Upon initial pupation, the pupa is orange without any dark brown and cream-colored patches, and then darkens within 24 hours. The mature pupa is nearly entirely dark brown with a small amount of cream colored mottling. The cremaster is black. Dorsally the head is mainly cream colored with dark brown spots where the eyes are located. Antennae are found ventrally between the wings, and they have alternating patches of dark brown and cream-colored patches. Ribbed portions of the antennae are visible. The two halves of the proboscis are found in between the antennae and are nearly entirely dark brown. Where the proboscis meets the head ventrally there is a dark brown bean shaped patch on the head. Posterior to the head, there are two raised cream-colored diamond-shaped tubercles dorsally. The thorax dorsum is raised and keel-shaped with an additional raised line down the midline. Dorsally the abdomen has alternating dark brown and cream-colored roughly triangular patches. The abdominal segments have two dorsal rows of tubercles on each side of the midline. These tubercles form the points of the dark brown triangular patches anteriorly. The dorsal anterior brown triangular patches vary in size and shape between individuals; the brown anterior triangular patches are sometimes joined to smaller brown triangular patches posteriorly with the cream-colored triangular patches in between these giving them a more pentagonal rather than triangular shape. Dorsally, along the midline within the cream colored central triangular patch of each abdominal segment, there are two dark brown circles in between the two dorsal tubercles. Smaller dark brown tubercles are found both superior to and inferior to the spiracles on segments A1-A8. Lateral and ventrolateral portions of segments A4-A6 have a dark brown band where the segments articulate. The wing case is dark brown with small amounts of cream-colored patches around the border of the wings, with some patches in the middle as well.

Differentiation of sympatric *Speyeria* larvae

Overall our observations indicate that *S. adiaste* can be distinguished from both *S. callippe* and *S. coronis* in the 4th, 5th and 6th instar by the presence of mottled brown patches on the head capsule. We did not observe discernible differences in larval color

pattern among *S. a. clemencei*, *S. c. comstocki* and *S. c. coronis* in the 2nd or 3rd instars. In the 4th instar the body of *S. a. clemencei* appears to be darker and less strongly marked than *S. c. comstocki* and *S. c. coronis*, which have brighter colored body mottling overall, as well as whitish-orange patches near the dorsal scoli (*S. c. coronis* has more orange colored patches near the dorsal scoli compared to *S. c. comstocki*) and a more prominent gray to whitish mottled lateral band between the dorsolateral and lateral scoli. In the 5th instar *S. adiaste* and *S. callippe* resemble one another in body coloration too much to differentiate them, with both being fairly darkly colored. *S. c. coronis* 5th instars look very similar to their 4th instars, which makes it easy to distinguish it from the other two species in this stage. In the 4th and 5th instar *S. a. clemencei* develop mottled brown patches on the lateral and dorsolateral area of the head capsule that are absent in *S. c. comstocki* and *S. c. coronis*.

In the sixth instar larvae of all three species can be well-distinguished morphologically. Of the 14 characters examined, the extent and brightness of orange coloration at the base of the dorsal and dorsolateral scoli, the presence/absence of brown/tan patches on the vertex of the head capsule and the color of the setae on the scoli serve to separate the species (see Table 1 and Fig. 3). *S. a. clemencei* consistently had the darkest colored dorsal and dorsolateral scoli (Fig. 3) and was the only one of the three species to have the brown/tan colored patches on the vertex of the head capsule. In contrast, *S. c. coronis* was brighter overall in coloration and had much more orange coloring in both the dorsal and dorsolateral scoli, and was different in that it had brown colored setae on the scoli, compared to black setae for the other two species. *S. c. comstocki* was intermediate in coloration and similar to *S. a. clemencei* in that it appeared dark overall, however it consistently had more orange coloration in its scoli (see Table 1 and Fig. 3).

Adult biology

S. adiaste clemencei individuals were observed nectaring on five different nectar sources on Chews Ridge during the 2012 and 2013 flight seasons (Fig. 4). Combining both years, 68% (82 of 120) of the individuals nectared on *Cirsium occidentale* (Asteraceae), and 28% (33 of 120) nectared on *Monardella villosa* (Lamiaceae). The remaining nectaring records included *Asclepias eriocarpa* (Asclepiadaceae; 3 of 120; 3%); *Verbena lasiostachys*, (Verbenaceae; 1 of 120, 1%); and *Wyethia helenioides* (Asteraceae; 1 of 120; 1%). We observed a marked shift in the frequency of the two main nectar sources used between 2012 and 2013 (Fisher's Exact Test, $p = 0.0004$). In 2012, *C. occidentale* made up 78% (72 of 92) of the observations, compared with 37% (10 of 28) in 2013. *M. villosa* comprised 20% (18 of 92) of the nectar records in 2012, compared with 55% (15 of 28) in 2013. There were no observations of *S. adiaste* adults nectaring on *V. lasiostachys* or *W. helenioides* in 2012 (Fig. 4).

Table 1. Variation in the key diagnostic characters for sixth instars of three sympatric *Speyeria* species at Chews Ridge. N = # of larvae used for the description. Other characters studied were too variable to allow reliable determination.

Character	*S. adiaste clemencei*	*S. callippe comstocki*	*S. coronis coronis*
T1-T3 Dorsal Scoli	Basal 0-40% pale tan to dark brown; Apical 60-100% black. N = 12	Basal 50% gray-brown to brownish-orange; Apical 50% black. N = 8	Basal 50-65% pale gray to orange; Apical 35-50% black N = 1
A1-A10 Dorsal Scoli	Basal 0-40% pale tan to dark brown; Apical 60-100% black. N = 12	Basal 50-80% gray-brown to brownish-orange; Apical 20-50% black. N = 8	Basal 80% pale-orange to orange; Apical 20% black. N = 1
A1-A8 Dorsolateral Scoli	Basal 20-25% brown to dark brown; Apical 75-80% pale yellowish-brown to orange-brown grading into black. N= 12	Basal 80% yellowish-orange to orange; Apical 20% black. N = 8	Basal 80% orange; Apical 20% black. N = 1
Brown/Tan Patches on Vertex of Head Capsule	Present, head capsule is black with narrow patches of tan/brown present on vertex and dorsolaterally N = 12	Absent, head capsule is all black. N = 5	Absent, head capsule is all black. N = 1
Setae on Body Scoli	Black N= 12	Black N= 5	Brown to dark brown. N= 1

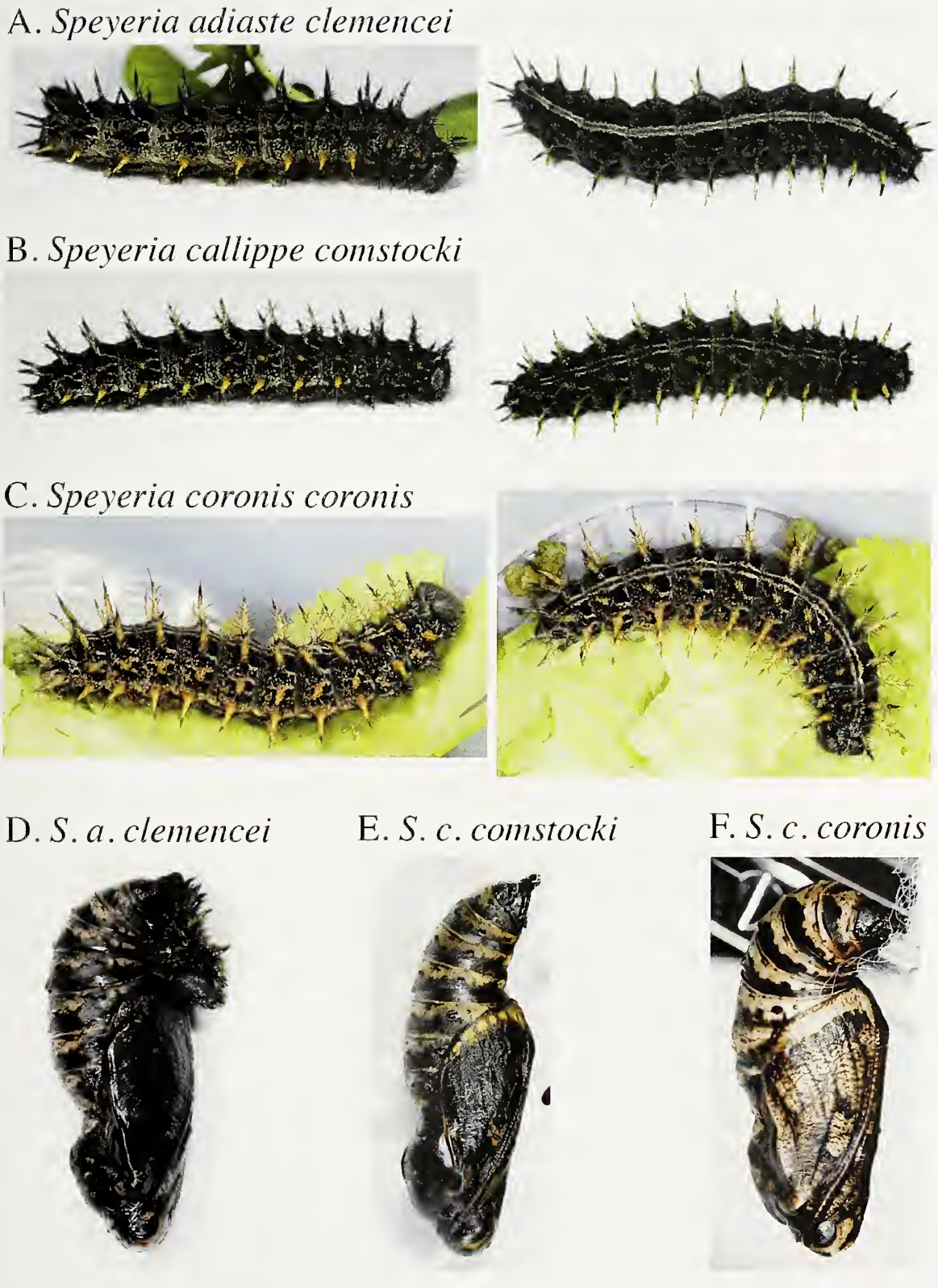

Figure 3. Ultimate instar (6th) and pupa of the three *Speyeria* species found at Chews Ridge, Monterey Co., CA. On the left side (A-C) is the lateral view and right side is the dorsal view. (A) *S. a. clemencei.* (B) *S. c. comstocki.* (C) *S. c. coronis.* In the 6th instar, *S. a. clemencei* can be distinguished by its darkly colored dorsal and dorsolateral scoli compared with the other two species. (D-F) are lateral views of *S. a. clemencei, S. c. comstocki* and *S. c. coronis* respectively. *S. adiaste* and *S. callippe* are quite similar in pupal morphology.

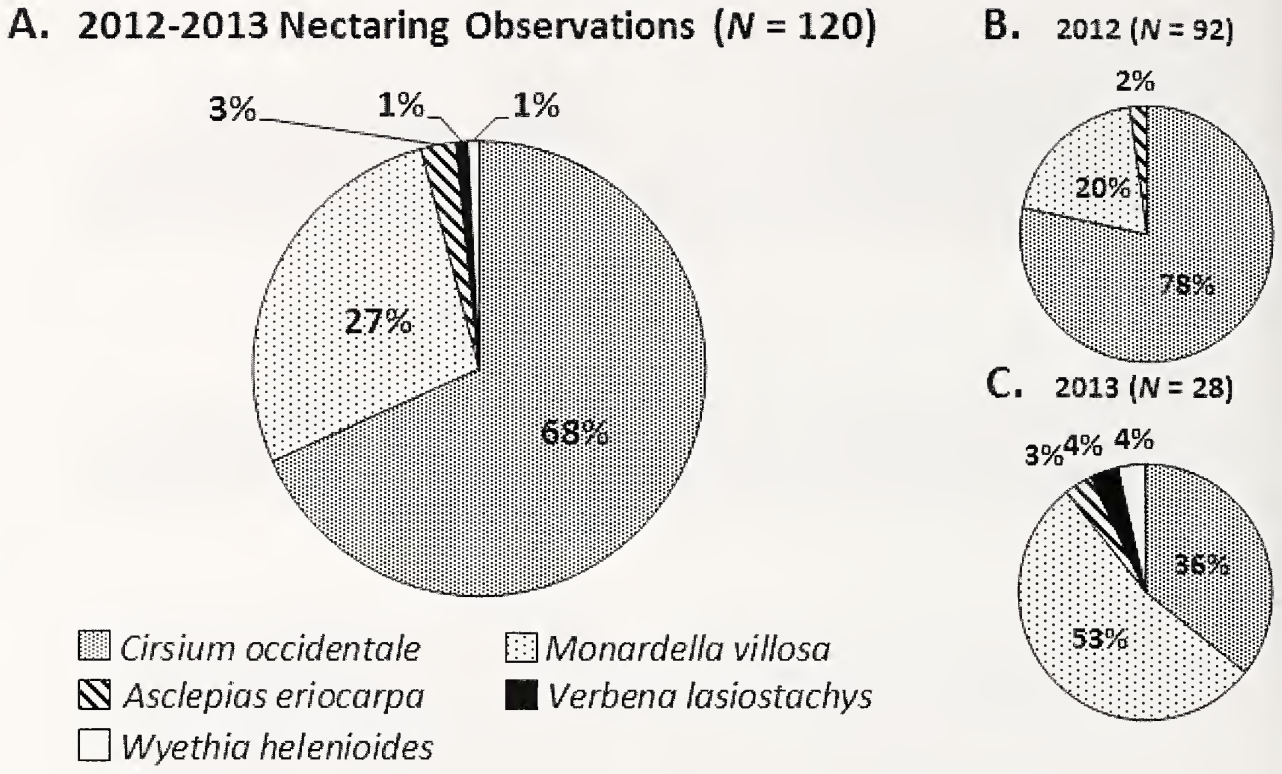

Figure 4. Adult nectar observations on Chews Ridge. (A) Combined data from 2012-2013 (N = number of observations). (B) Nectaring observations from 2012 only. (C) Nectaring observations from 2013 only. Note the relative difference in *Cirsium* and *Monardella* between years.

We observed a significant difference in behavior between males and females (Fisher's Exact Test, $p < 0.0005$; Fig. 5). The most common male behaviors were search flight (39%, 196 of 506), direct flight (28%, 143 of 506), and nectaring (20%, 101 of 506). Females were also often observed in search flight (34%, 42 of 125), however only 4% (5 of 125) in direct flight, and 13% (17 of 125) nectaring. Females were observed perching (49%, 61 of 125) more than males (7%, 37 of 506). One male behavior not observed in females was chasing other butterflies and insects, occasionally even hummingbirds (6%, 29 of 506).

Ovipositing females typically landed near areas with the host plant *V. p. quercetorum*. Upon landing, a female dragged her abdomen along the ground, simultaneously shivering and flapping her wings as she walked. Females seemed to "false oviposit" quite often, as suggested by an extreme curling of their abdomen as if probing for the host plant or appropriate substrate. Females spent most of their time near senescing *Viola*. They walked under broken branches and twigs, into ground squirrel holes, under non-host plants, and under leaf debris to oviposit one egg at a time. The females seemed to prefer shadier areas with dappled sun under and near deciduous and non-deciduous oaks (*Quercus* spp.). Females appeared to begin ovipositing right away during their relatively short time on the wing, indicating an absence of reproductive diapause, in contrast to sympatric *S. coronis coronis* (R. Hill & C. Tenney, pers. observ.; Shapiro & Manolis, 2007.) and *S. coronis snyderi* in northern California (Sims, 1984).

Discussion

The goal of this study was to document larval morphology, ecology, and other aspects of the biology of *S. adiaste*, to lay the groundwork for further studies and to help inform conservation decisions regarding this species. Given that *S. adiaste* is sympatric with two other *Speyeria* species, simply finding and correctly identifying a *S. adiaste* larva in the field is an important first step. In addition, a better understanding of the life history traits and natural history of *S. adiaste* can identify the most critical aspects of its biology to guide conservation efforts. It is our hope that this study encourages study of immature stage biology and demonstrates the importance of natural history observations for increasing the understanding of species of conservation concern.

Larval morphology and ecology

One challenge to conducting fieldwork on larval ecology is species identification. Previous authors have stated that *S. callippe* and *S. adiaste* larvae are difficult to distinguish (Scott, 1986; Dunford, 2009; Allen *et al.*, 2005). Our results indicate that *S. adiaste* and *S. callippe* larvae are very similar, however, based upon examining 14 characters, we found that *S. a. clemencei* can be separated from the other two species in the 4th and 5th by head capsule color, and all three species can be distinguished in the 6th instar by coloration of the scoli, coloration of setae on the scoli, and the presence/absence of brown/tan patches on the head capsule (Table 1, Fig. 3). The lack of clear color pattern differences in 2nd to 3rd instars between species hampers field identification and indicates rearing to at least the 4th instar is necessary for correct determination. However, additional traits other than color pattern could be studied for differences, but their utility would be limited for field identification as a microscope will likely be needed. The characters useful for separating 4th, 5th, 6th instar *S. adiaste*, *S. callippe* and *S. coronis* observed here are consistent with Comstock and Dammers (1931, p. 44) observations on morphological differences between 6th instar larvae of *S. a. atossa*, *S. callippe macaria*, and *S. coronis semiramis*. However, the "more pronounced lateral mottled gray line" that Comstock and Dammers (1931) used to distinguish *S. a. atossa* from *S. c. macaria* and *S. c. semiramis* appears variable within *S. a. clemencei*, making it a less useful diagnostic character to separate 6th instars on Chews Ridge. In the 6th instar the majority of the *S. a. clemencei* individuals had a prominent gray mottled band between the dorsal and dorsolateral scoli, but a few were weak, and some very weak. There was less variation within *S. callippe comstocki* for this character, with the general trend being that the gray mottled band was much less conspicuous in most larvae, however, one individual did have a prominent gray band. In the 6th instar *S. coronis coronis* appears distinct from the other two species on Chews Ridge in having a grayish band with orange/tan mottling.

Despite the utility of the coloration of the dorsal and dorsolateral scoli for identification, there was variation in these characters within broods and among broods. Given our relatively small sample size, additional observations may be needed to confirm the overall pattern of differences in these characters among the sympatric species. Two broods of *S. a. clemencei* larvae were used for the morphological description, with two broods for *S. c. comstocki* and one brood for *S. c. coronis*. Furthermore, only a single specimen of *S. c. coronis* was used for the morphological comparisons. A larger sample size from multiple broods for *S. c. coronis* may be needed to understand phenotypic variation within this species; however, due to the strong morphological distinction between *S. a. clemencei* and the *S. c. coronis* larva, it does not seem likely that the *S. c. coronis* larval phenotype will broadly overlap with further observations. In contrast, *S. a. clemencei* and *S. c. comstocki* larvae were more similar, and variation within and among the *S. c. comstocki* brood and *S. a. clemencei* broods may make it challenging to identify these two species in the field (see Table 1 for details on variation). Although we were confident in using the dorsal and dorsolateral scoli as a means of separating 6th instar *S. a. clemencei* and *S. c. comstocki*, further observations with multiple broods would help provide a more complete understanding of variation in the immature stages of

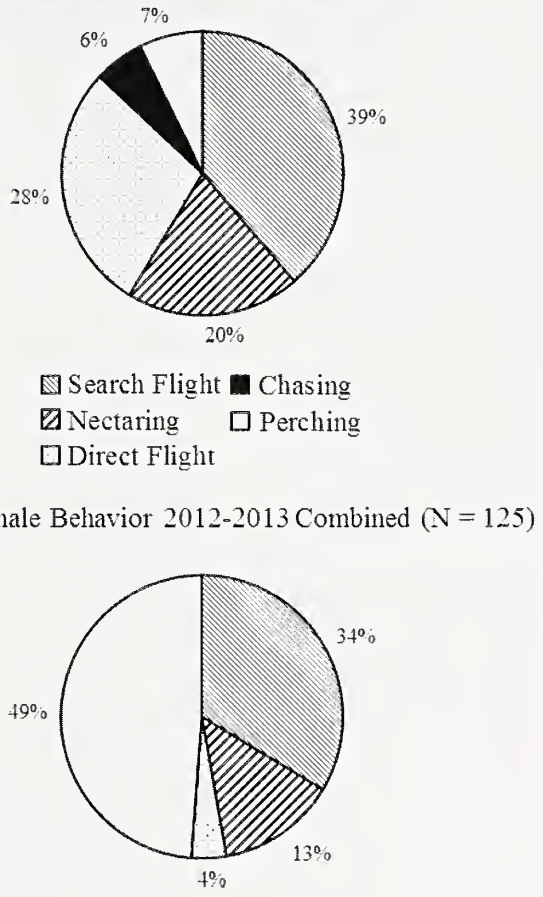

Figure 5. Adult behavior on Chews Ridge. (A) Observed male behaviors combined from 2012-2013. (B) Observed behavior for females during 2012-2013. N = total observations, not necessarily unique individuals.

these two species. The fact that Comstock and Dammers (1931) identified similar characters for differentiating between *S. a. atossa* and *S. callippe macaria* (and *S. coronis semiramis*) suggests that the traits are broadly useful between species (see also Sims *et al.*, 1979).

Although some instars of *S. adiaste* can be separated from sympatric *Speyeria* on Chews Ridge, additional field studies of *S. adiaste* larvae are challenging due to difficulties in locating the larvae. *Speyeria* larvae are known to be difficult to find in the field, possibly due to their nocturnal feeding habits (Shapiro & Manolis, 2007; Allen *et al.*, 2005; Comstock, 1927) and our results searching for *S. adiaste* are consistent with this. On May 1st, 2012, we searched areas with dense *Viola purpurea quercetorum* host plant on Chews Ridge from 19:30-22:30 (three observers = nine person-hours total search time). Based on the date of appearance of the first adults in 2012, and using the average duration of each instar based on lab rearing (see Results), most larvae in the population should have been in the 4th instar by this date, indicating they should have been fairly conspicuous during the search. However, we only found a single 4th instar larva with this effort. Furthermore, if larvae were easy to find, they should have been encountered in April and May of 2013 during an intensive survey of *V. purpurea* abundance on Chews Ridge. The survey was conducted during daylight hours and we did not find a single larva in 270 person-hours spent counting *V. purpurea*, despite commonly finding evidence of herbivore damage on the leaves. It is possible that larvae were overlooked during the survey in early April when they would have been very small 1st/2nd instars, which would have been very difficult to find due to their size and coloration. However, by mid-May, larvae should have been in the 6th instar, and therefore, much more conspicuous during the survey effort. Overall, our observations suggest finding larvae in the field is challenging, making field studies of larval ecology particularly difficult.

The difficulty finding larvae in the field places a premium on captive rearing of this species in order to better understand its larval ecology. Our studies indicate that *S. adiaste* can successfully be reared in the laboratory by using commercially available *Viola* spp. (*V. tricolor* and hybrids). For a rare butterfly species such as *S. adiaste*, successful rearing in the lab allows for the possible implementation of a captive rearing program for restoration purposes. Currently there are many organizations that are involved in rearing rare and endangered butterfly species for reintroduction into the wild (James & Nunnallee, 2011). Captive rearing has been shown to be a robust method to maintain severely at-risk populations in the short-term, though it does not appear to be a viable long-term solution (Crone *et al.*, 2007). We loosely followed rearing methods described by Mattoon *et al.* (1971) and Wells *et al.* (2011). However, there was likely room for improvement in our rearing methods that could have led to increased oviposition. During our rearing, we obtained 70 ova from a wild-caught *S. adiaste* female captured late in the season. We used only a single full-sized grocery bag during oviposition, in contrast to Wells *et al.* (2011) method of replacing the bag each day; this could have limited the number of eggs laid. Furthermore, although females were given 7% honey-water solution and fed *ad libitum* in lab, no amino acids were added. The addition of amino acids could promote increased oviposition by supplementing sub-optimal larval food resource conditions the females might have encountered in the wild (Mevi-Schutz & Erhardt, 2003; Wagner *et al.*, 1997).

The life history strategy and larval ecology of *S. adiaste* have important implications for the persistence of its populations. *Speyeria* butterflies appear adapted to areas with dense, well-connected patches of their *Viola* host plant, where larvae can quickly locate a host and roam from plant to plant, and larval mortality is mitigated by high fecundity. *Speyeria* females oviposit single eggs on various substrates near dry or senescing violet host plants, but very rarely oviposit on *Viola* itself (Wagner *et al.*, 1997; Kopper *et al.*, 2000). The overwintering larvae do not eat their host until the following spring, and must locate suitable host after months of diapause, making this a critical step for larval survival. Increased larval mortality is likely if patches of host plant are small, patchily distributed or do not have adequate density. Laying large numbers of eggs is likely a strategy to overcome the high mortality rate of overwintering first instars (Wagner *et al.*, 1997; Kopper *et al.*, 2000). *S. idalia* and *S. diana* may represent the upper limit of fecundity within *Speyeria*, laying from 1000 eggs to occasionally over 2000 eggs in their lifetime, some of the highest amounts ever recorded from butterflies under laboratory settings (Wagner et al., 1997; Wells *et al.*, 2011). Our observations suggest that this level of fecundity probably does not occur in *S. adiaste*, which likely lays several hundred eggs, rather than thousands. This is more consistent with other *Speyeria* species that have been shown to oviposit between 100-1000 eggs (James & Nunnallee, 2011). The lower fecundity of *S. adiaste clemencei* suggests mortality of overwintering larvae is a critical aspect of population persistence. Overall, the effectiveness of this life history strategy appears to be limited for many current *Speyeria* populations, since many members of this genus are reportedly suffering due to habitat degradation and perturbations of their *Viola* host plants (Hammond & McCorkle, 1983).

Habitat loss and changes in *Viola* density and distribution likely present difficult challenges for *Speyeria* larvae, which are limited in their ability to detect and find host plants (Bierzychudek *et al.*, 2009). In a field-based study of host-finding capabilities of endangered *Speyeria zerene hippolyta* larvae, Bierzychudek *et al.* (2009) found that larvae were unable to distinguish their host plant from non-host at distances of three centimeters. Weiss and Murphy (1988) provide further evidence of the difficulties faced by early instars in their attempts to find food. Their model, using simulations of a rough-textured grassland environment, indicated that one to two millimeter sized caterpillars, similar in size to first instar *Speyeria* larvae, would need to walk 42 meters in order to cover one meter of linear distance. These results may be particularly important for *S. a. clemencei* on Chews Ridge, as their host *Viola purpurea* is abundant, but very patchily distributed (K. Zaman, C. Rush, C. Tenney, R. I. Hill, unpublished). This patchy distribution may make it difficult for first instar *S. adiaste* to locate their host plant if they have similar host finding abilities to those of *S. z. hippolyta*. As suggested by the studies of Bierzychudek *et al.* (2009) and Wagner *et al.* (1997), in order to counteract the lack of efficient host finding capabilities of *Speyeria* larvae and the oviposition behavior of females, dense patches of their *Viola* host plant are necessary for larvae to successfully find food and prevent starvation. In order to better understand the possible restoration requirements for *S. adiaste*, we recommend study of larval movement patterns and host finding abilities and their relation to the abundance and distribution of their *Viola* host plant.

Habitat and host plant

In order to obtain a clearer view of *S. adiaste* across its range, it is useful to compare our observations for *S. a. clemencei* with available information on the habitat and host plant requirements for the remaining subspecies. Across the range of *S. adiaste*, there appears to be a trend toward higher elevation and more open habitats southward (Scott, 1986). Scott (1986) suggests that the northern *S. a. adiaste* inhabits redwood forest openings, which corresponds with Comstock's (1927) description of *S. a. adiaste* as a "forest lover, delighting in the flowered glades adjacent to the redwood groves." Howe (1975) corroborates that *S. a. adiaste* is "more closely associated with forests than either *clemencei* or *atossa*." *S. a. clemencei* at Chews Ridge inhabits mixed oak-pine woodland. Hovanitz (1970) describes the habitat of *S. a. clemencei* as "openings in oak woodland," and indicates that both *S. a. adiaste* and *S. a. atossa* share this same type of habitat. *S. a. atossa* was apparently more similar to *S. a. clemencei* in that it inhabited more open habitats (Howe, 1971; Comstock, 1927).

We observed *S. a. clemencei* on Chews Ridge using *Viola purpurea quercetorum* (Baldwin *et al.*, 2012) as its host plant. Chews Ridge females were observed ovipositing near *V. p. quercetorum* growing in shaded areas under oaks. In comparison, *Viola pinetorum* has been listed as a possible host plant for *S. a. atossa* (apparently misspelled "*pinctorum*" Comstock 1927, p. 89), due to its abundance in fields with emerging *S. a. atossa* adults (Comstock, 1927). The host plant for *S. adiaste adiaste* is unclear; Scott (1986) and Robinson *et al.* (2002) suggest *Viola ocellata*, one of several *Viola* species found within the range of *S. a. adiaste*.

Adult ecology

Behavioral differences between sexes is common among adult Lepidoptera, which can lead to differential use of habitat and adult resources (Scott, 1986). There was a clear difference in the observed behavior of male and female *S. a. clemencei*. Males flew faster and higher above the ground, and were thus more visible to observers. Females typically flew low to the ground and were constantly landing and searching. Males were also observed relatively more often in flight-related activities (Fig. 5), increasing the probability of encounters with the observers. Male observations involving flight (search flight, direct flight, and chasing) comprised 73% (368 of 506) of male observations, compared with 38% (47 of 125) flight-related observations (search and direct flight) for females (Fig. 5). Males were most commonly observed in search and direct flight, presumably to locate females (Fig. 5). Females were most commonly observed in behaviors related to finding host plants and oviposition, as perching and search flight made up 49% and 34% of our female behavioral observations respectively.

It is important to note that the calculated percentages for each observed behavior may not reflect the relative amount of time that males and females were engaged in that particular behavior outside of our observation periods. The conspicuousness of flight behaviors indicates that this alone could cause these behaviors to be highest in frequency. Still, even among conspicuous flight behaviors there were relative differences between males and females, such as little direct flight and no chasing in females, that is indicative of differences between the sexes. Furthermore, if our observations were simply the result of detecting the most conspicuous behavior, such as those involving flight, then perching should not have been as commonly observed in females. If we assume the frequency we observed for each behavior was constant over the entire time that the butterflies were active during the day, the increased

time in flight for males may be associated with increased predation risk, and higher-energy expenditure. The relatively less conspicuous behavior of females likely keeps them out of sight of predators, and may conserve energy for egg production.

In addition to host plant ecology, adult nectar sources may play an important role in population persistence of *S. adiaste*. Published nectar plant records of *S. adiaste* include various thistles (*Cirsium*, *Carduus*, and *Silybum*), tansy ragwort (*Senecio jacobea*), and *Brodiaea* (Shapiro & Manolis, 2007). For *S. a. clemencei*, Howe (1975) listed *Aesculus californica* and *Eriodictyon californicum* as nectar sources. These plants are not present at the Chews Ridge study site, but rather are found at lower elevations nearby (R. Hill and C. Tenney pers. obs.). On Chews Ridge, *S. a. clemencei* was observed nectaring on five different plant species (Fig. 4). *Cirsium occidentale* and *Monardella villosa* were the most visited flowers during both years. *C. occidentale* was used heavily within the first half of the flight season, after which the majority of the thistle on Chews Ridge senesced in 2012 and 2013. *M. villosa* blooms later in the flight season, and was used throughout the remainder of the season in 2012 and 2013. Interestingly, despite the relatively high abundance of *Asclepias eriocarpa* and *Verbena lasiostachys* in both 2012 and in 2013, *S. adiaste* adults very rarely nectared on those flowers. This indicates a preference for *C. occidentale* and *M. villosa* compared with the other three nectar sources.

Although *C. occidentale* appears to be the most commonly used nectar source, we observed between-year variation in nectar source use that points to the importance of availability of multiple nectar species for population persistence. In 2012 *C. occidentale* made up the majority of all nectaring observations (78% of 92 observations). However, in 2013, there was a shift toward *M. villosa* as the main nectar source (55% of 28 observations). The 2013 flight season was a year of low adult *S. a. clemencei* abundance on Chews Ridge, despite abundant *Viola*, probably linked to lack of rainfall (K. Zaman, C. Tenney, R. I. Hill, unpublished data). Lack of rainfall also seems to have affected the nectar source *C. occidentale* during 2013, as it senesced relatively early compared with 2012, and was unavailable as a major nectar source by mid to late June. As a result, *M. villosa* became the primary nectar source throughout the mid to late part of the flight season when females were ovipositing. Studies done with *Speyeria mormonia* have shown that fecundity declines in direct proportion to declines in adult diet (Boggs & Ross, 1993). Assuming this holds for *S. adiaste*, the lack of an alternate nectar source on Chews Ridge later in the season in drought years could severely limit the number of eggs oviposited, and thereby limit the number of overwintering larvae. This suggests that the presence of at least two nectar sources on Chews Ridge, or a preferred nectar host that persists throughout the flight season (in other localities), may be a critical factor in allowing the population to persist during drought, especially prolonged drought. Drought conditions may also have a compound effect on populations across years by causing relatively higher mortality rates among overwintering larvae, as well as reduced female fecundity in the same season, which would consequently leave fewer overwintering larvae to make it through the subsequent dry spring. Further study of ecological factors leading to population declines in this and other *Speyeria* species are clearly warranted.

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